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New Strategies for Prevention and Treatment of Insect Bite Hypersensitivity in Horses

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Keywords Insect bite hypersensitivity · Allergens · Allergen-specific immunotherapy · eIL-5-VLP · IL-31-VLP therapeutic vaccines

Introduction

Equine insect bite hypersensitivity (IBH), also known as sweet itch, Queensland itch, summer eczema, or Kasen is the most common allergic skin disease of horses [1] and is clinically manifested as a chronic relapsing seasonal dermatitis [reviewed in 2]. IBH is initially presented as pruritic dermatosis frequently affecting the mane and tail area followed by self-trauma leading to hair loss and excoriations, which contribute to development of secondary bacterial infections [2–4]. It is caused by bites of insects of the genus *Culicoides*, known as biting midges [5, 6]. The prevalence correlates with geographical distribution of the midges [7], i.e. depends on environmental factors [8, 9] as well as on genetic factors, such as breed, lineage or family [reviewed in 2]. IBH has been described worldwide, except in Iceland, and affects approximately 10% of horses of all breeds [7].

While feeding, the midges inject a pool of various salivary gland proteins leading to sensitization and allergy in predisposed horses [10, 11]. Studies in humans have shown that during the sensitization process, IL-25, IL-33, and thymic stromal lymphopoietin (TSLP) are released from inflamed or injured epithelial cells, inducing type 2 innate lymphoid cells (ILC2) to produce IL-5 and IL-13 [12, 13]. Under the influence of these cytokines and TSLP, antigen-presenting cells (APCs) direct the immune response towards Th2 with production of IL-4 and IL-13. Subsequently, B cells undergo class switch and produce allergen-specific IgE that binds to high affinity FcεRI receptors on basophils and mast cells, leading

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to sensitization (Fig. 1A) [14, 15]. In sensitized, allergic individuals, re-exposure to the allergen causes cross-linking of IgE bound to high-affinity FcεRI receptors on mast cells and basophils and subsequent immediate release of effector cell mediators. Vasoactive amines, lipid mediators, granule enzymes, and cytokines cause the clinical signs of immediate hypersensitivity (Fig. 1B). Late-phase reaction occurs 2–4 h after the exposure with a peak after 24 h. The release of inflammatory mediators by the activated mast cell causes infiltration of leukocytes, mostly Th2 cells and eosinophils at the site of the allergic reaction (Fig. 1B) [14, 15].

Similarly to human allergy, various studies in horses have shown an imbalance between a Th2 and T regulatory (Treg)/Th1 immune response in IBH [16–19], with a relative increase of the Th2 response. The involvement of IgE-mediated, type I hypersensitivity reactions to *Culicoides* allergens with activation of mast cells, followed by a late-phase reaction characterized by infiltration of lymphocytes and eosinophils is well established [reviewed in [7, 20, 21]. Few studies suggest a possible involvement of delayed-type hypersensitivity (DTH; type IV) in the pathogenesis of IBH [22]. Type IV hypersensitivities can be further divided into types IVa, IVb, IVc, and IVd based on predominant cell types. Type IVb of DTH in drug hypersensitivity, is strongly associated with IL-5 producing Th2 cells and eosinophilia [23]. Eosinophils are thus a common feature of both late-phase reaction of type I hypersensitivities and type IV hypersensitivities. Moreover, they seem to play a crucial role in IBH, as the allergic lesions are often characterized by infiltration with eosinophils [7] and eosinophilia is associated with severity of IBH [24••]. Furthermore, studies by Fettelschloss-Gabriel et al. indicate an important role for IL-5 in the pathogenesis of IBH [24••, 25••]. IL-5 is a cytokine that displays multiple effects on eosinophils and affects their differentiation, migration, activation and survival. Once activated by IL-5, eosinophils release granule enzymes and effector molecules such as leukotrienes and

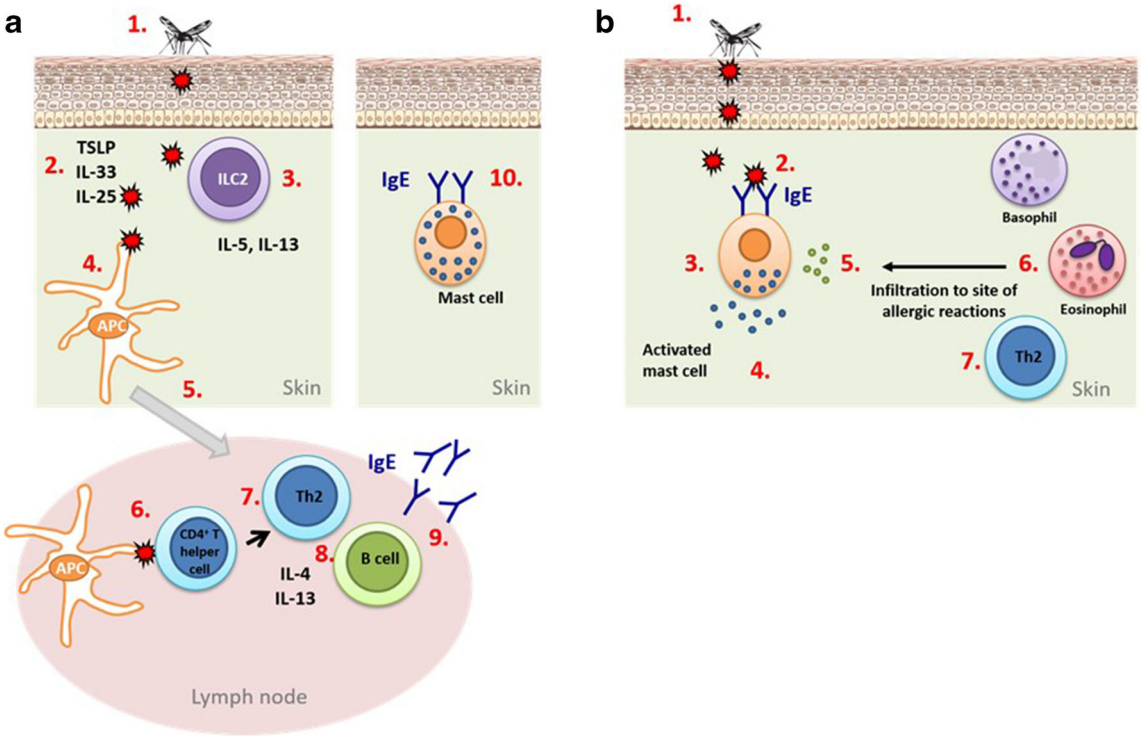

Fig. 1 Simplified scheme of type I hypersensitivity reaction as thought to occur in insect bite hypersensitivity. Bold in the fig. legend indicates mechanisms that have been demonstrated to be involved in IBH. (**A**) Sensitization. (**1**) *Culicoides* midges bite horses, injecting saliva that contains a variety of proteins. (**2**) Upon inflammation or damage, the epithelium produces **TSLP**, IL-33, and IL-25. These cytokines act as alarmins and activate group 2 innate lymphoid cells. (**3**) ILC2s secrete IL-5 and **IL-13**. (**4**) APCs take up salivary gland proteins and are modulated by the ILC2 cytokines. (**5**) APCs migrate to the draining lymph node. (**6**) APCs present the allergen peptides on MHC class II to naïve $CD4^+$ T helper cells. (**7**) Naïve $CD4^+$ T helper cells differentiate into T helper type 2 cells (**Th2**). (**8**) Th2 cells produce **IL-4** and **IL-13** that instruct the B cells to undergo class switching. (**9**) Upon class switching, B cells produce allergen-specific **IgE**. (**10**) IgE binds to the high affinity IgE receptor (FcεRI) on mast cells thus sensitizing the horse to *Culicoides* allergens. (**B**) Re-exposure: immediate and late-phase reaction. (**1**) Upon re-exposure to *Culicoides* allergens, (**2**) the allergens bind to the mast cell–bound allergen-specific IgE antibodies, causing cross-linking between the receptors. (**3**) Mast cells are thus activated and release inflammatory mediators, such as **histamine**, **leukotrienes**, prostaglandins, and PAF, (**4**) leading to development of edema, erythema, and itch, within minutes of allergen re-exposure. (**5**) Activated mast cells also release chemokines and cytokines responsible for the recruitment of effector cells to the allergy site. (**6**) These effector cells are mainly **eosinophils**, **Th2** cells, and basophils, causing the late-phase reaction, which starts 2–4 h after exposure and reaches its peak at 24 h. (**7**) Th2 cells produce cytokines, in particular IL-5 that further recruits **eosinophils** to the site of allergic inflammation

major basic protein that in turn trigger degranulation of mast cells and basophils, thus forming a vicious circle of allergic inflammation.

Although IBH is the most common skin disease of horses, the only efficient treatment available is, beside reduction of exposure to *Culicoides* by stabling, use of blankets or repellents, symptomatic therapy using glucocorticoids [7, 26]. Antihistamines seem to be of limited efficacy in the treatment of IBH [27]. In this review we will highlight the most recent developments in preventive options and treatments for the equine IBH.

Allergen-Specific Immunotherapy

Allergen-specific immunotherapy (ASIT) is the only allergy modifying treatment for type I hypersensitivities [28] and has been shown to reduce progression of the disease as well as to decrease the risk of development of new allergic conditions in atopic patients [29]. ASIT has been used for therapy of human allergies for over 100 years and was first applied by Noon et al. who treated hay fever patients with pollen extract [30]. The mechanism underlying ASIT is thought to be a shift of the immune response from Th2 towards a regulatory immune response. In response to ASIT, IgG antibodies are produced, in particular IgG4 that block the binding of allergen-specific IgE antibodies to the allergens. Additionally, ASIT is associated with decreased production of IL-4 and IL-5, the signature cytokines of Th2 $CD4^+$ T cells [31], an increase of the allergen-specific Treg with the production of regulatory cytokines, IL-10 and TGF-β [32, 33], as well the induction of allergen-specific Breg [34].

ASIT has proven to be relatively inexpensive, highly effective, and long-lasting when high-quality antigens are used [35]. Allergen extracts have been used with a good outcome. However, the success of therapy depends on the quality of the extracts used as reviewed in Zhernov et al. On top of that, standardization of allergen extracts is difficult. They can vary greatly in the amount, potency and immunogenicity of individual allergens and major allergens can be lacking [36]. As IgE-mediated type I allergies are a global health problem affecting around 30% of human population in industrialized countries, it is vital to further develop ASIT with focus on safety and clinical improvement using well-defined standardized allergens [36]. The only way forward is by means of molecular applications, and presently different strategies to produce recombinant allergens are in development [35, 36] with the aim to reduce their allergenicity but maintain immunogenicity [37].

Another approach to control allergies is the use of preventive immunotherapy, which can be applied in high-risk individuals before sensitization occurs, or in individuals with increased allergen-specific IgE before development of clinical signs [38]. To date, there are only few studies on preventive immunotherapy. Campana et al. showed that vaccination of non-allergic adults with recombinant hypoallergen derivatives of the major birch pollen allergens, Bet v 1 in Alum decreased the risk of developing birch allergy [39•].

Allergen-Specific Immunotherapy in Equine IBH

To our knowledge, three attempts to treat IBH with ASIT have been published [40–42]. All three have used whole body extract (WBE) of *Culicoides* midges. These crude WBE consist of a mixture of hundreds of proteins and other substances, whereby the salivary gland proteins, i.e. the allergens for IBH, only represent a minute fraction of the extract. Furthermore, the extracts were not derived from the most abundant *Culicoides* species present in the environment of horses. This might be important as van der Meide et al. have shown that IBH-affected horses have higher IgE levels to midges caught in the environment of the horses as compared with laboratory-bred species [43].

In two placebo-controlled studies IBH-affected horses treated with *Culicoides* whole body extract (WBE) showed no significant improvement in clinical signs when compared with the placebo-control group [40, 42]. However, in both groups, clinical signs after vaccination improved when compared with signs before vaccination, probably because of a better insect control [42]. Anderson et al. obtained a significant improvement of clinical signs in IBH-affected horses treated with *Culicoides* WBE; however, a control group was not included [41]. The low efficacy of ASIT with insect WBE in early studies in humans [44, 45] as well as the controversial non-conclusive results of these studies show the necessity of using pure, well-defined allergens instead of crude WBE, ideally by first defining the sensitization profile of each horses. To achieve this goal, molecular approaches had to be applied for identification and production of *Culicoides* salivary allergens.

Culicoides Allergens in IBH

Over the last decade, extensive work has been put into identifying the causative allergens for IBH and producing them as pure recombinant proteins. Allergens from the three *Culicoides* species *C. nubeculosus* [46, 47], *C. sonorensis* [48], and *C. obsoletus* [49, 50] have been identified and produced. All of them have been expressed in *Escherichia coli*, some in insect cells and barley [51], and one in *Pichia pastoris* [52••] (Table 1). With a first available panel of allergens, Marti et al. have set up a protein microarray for detection of the IgE antibodies of IBH-affected horses [53•], which has now been improved with the addition of further *Culicoides* allergens, resulting in a total of 27 different *Culicoides* salivary proteins [54••]. This approach is an efficient method for the elucidation

Table 1 Overview of published *Culicoides* allergens

Allergens	*C. nubeculosus*	kDa	Allergens	*C. obsoletus*	kDa	Allergens	*C. sonorensis*	kDa
Cul n 1 [46]	Antigen 5–like protein	25	Cul o 1p [50]	Kunitz protease inhibitor	23	Cul s 1 [48]	Maltase	69
Cul n 2 [47]	Hyaluronidase	47	Cul o 2p [50]	D7-related salivary protein	18			
Cul n 3 [47]	Putative cystein endopeptidase	45	Cul o 1 [43]	Maltase	67			
Cul n 4 [47]	Secreted salivary protein	18	Cul o 2 [43]	Hyaluronidase	42			
Cul n 5 [47]	Secreted salivary protein	46	Cul o 3 [43]	Antigen 5–like protein	28			
Cul n 6 [47]	Secreted salivary protein	17	Cul o 4 [43]	Trypsin	27			
Cul n 7 [47]	Unknown salivary protein	21	Cul o 5 [43]	Unknown salivary protein	18			
Cul n 8 [47]	Maltase	69	Cul o 6 [43]	D7-related salivary protein	15			
Cul n 9 [47]	D7-related salivary protein	16	Cul o 7 [43]	Secreted salivary protein	15			
Cul n 10 [47]	Secreted salivary protein	48						
Cul n 11 [47]	Trypsin	30						

of the most relevant *Culicoides* allergens. It will simplify mapping of the sensitization pattern of IBH-affected horses, which in turn can serve as foundation for precision medicine–based ASIT.

Preventive and Curative ASIT

Having access to pure *Culicoides* allergens opens the possibility for development of both prophylactic and therapeutic ASIT. Ideally, preventive immunotherapy should be carried out prior to exposure to the allergens. Iceland is free of *Culicoides* that feed on horses, and therefore horses in Iceland do not suffer from IBH [55]. However, prevalence of IBH in Icelandic horses exported to *Culicoides* endemic area is high, as more than 50% of them get affected if not protected from the midges [56, 57]. Since Icelandic horses are only sensitized after the export, they offer a unique opportunity for development and study of preventive immunotherapy.

For development of prophylactic vaccine, not only the source of allergens and type of adjuvants have to be considered, but also the injection route may be of a great importance. Studies by Senti et al. in human grass-pollen allergic patients indicate that intralymphatic application of the allergens is more efficient and does not have more side effects than the subcutaneous route, commonly used in ASIT. Compliance to ASIT is often low because of the long duration of treatment and the many injections needed. The most important advantage of the intralymphatic application compared with the conventional treatment was that only 3 injections versus 54 were required.

With the aim to develop a prophylactic ASIT against equine IBH (Fig. 2), application route and adjuvants had first to be evaluated. Two vaccination experiments using pure r-*Culicoides* allergens were carried out in healthy Icelandic horses not exposed to *Culicoides*. The aim was to determine which immunization protocol would lead to the induction of a Th1/Treg immune response with production of IgG-blocking antibodies, an important feature of successful ASIT. In the first study, horses were immunized 3 times at 4-week intervals, receiving 10 μg of each allergen/injection either intradermally or intralymphatically. This study showed that the adjuvant IC31®, a TLR-9 agonist, was essential to induce a strong antibody response. The induced antibodies, mainly IgG1 and IgG4/7, were partly able to inhibit binding of IgE to the allergens, i.e. blocking antibodies were induced. Importantly, no allergen-specific IgE were induced by this prophylactic vaccination, indicating no risk of sensitization. The intralymphatic route resulted in a slightly stronger antibody response than the intradermal application [58••] and was used in the second experiment. Unfortunately, IC31® was no more available for use in horses; thus, in the second study, Alum alone, an adjuvant often used in ASIT, was compared with a combination of Alum and monophosphoryl lipid A (MPLA, a TLR-4 agonist). *Culicoides* r-allergens mixed in Alum alone and Alum/MPLA induced similar levels of allergen-specific IgG1 and IgG4/7 antibodies with strong blocking capacity. However, PBMC from horses vaccinated with Alum/MPLA, but not those from horses vaccinated with Alum only, produced significantly more IFN-γ and IL-10 upon allergen-specific *in vitro* re-stimulation as compared with unvaccinated control horses [59••]. To summarize, intralymphatic injections with small amounts of recombinant allergens in Alum/MPLA resulted in a Th1/Treg immune response with induction of high specific IgG antibodies levels, displaying a strong blocking capacity. This indicates that such an immunization protocol would be suitable for prophylactic ASIT. A challenge experiment needs now to be performed to know whether this type of prophylactic vaccination can protect Icelandic horses against IBH after exposure to *Culicoides*.

As an alternative to subcutaneous immunotherapy (SCIT), sublingual immunotherapy (SLIT) has also been shown to be

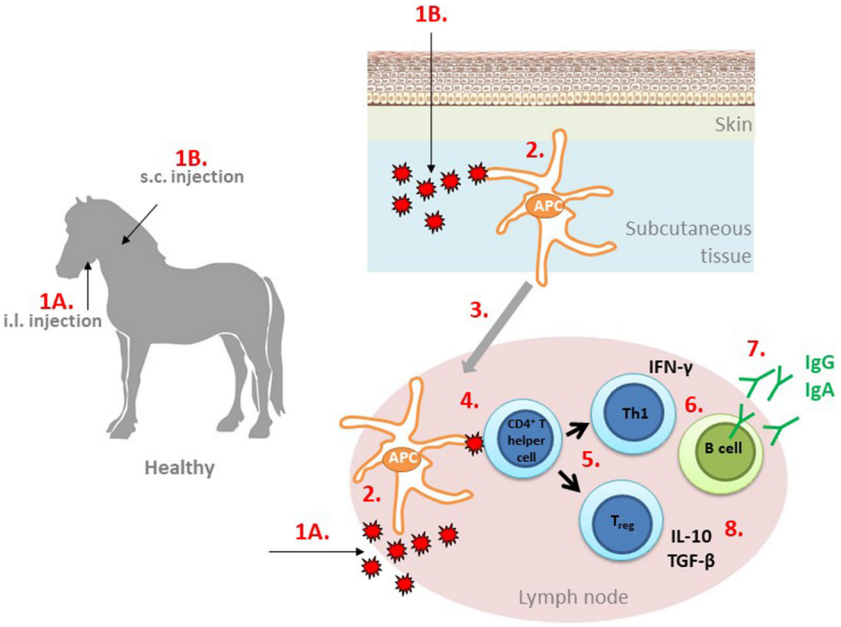

Fig. 2 Simplified scheme of preventive allergen-specific immunotherapy as described in this review. Immune reactions that have been demonstrated in horses are indicated as bold in the fig. legend. (**1**) The vaccine consisting of recombinant *Culicoides* allergens in adjuvants is injected directly into the submandibular lymph node (A) or subcutaneously (B) or intradermal. The advantage of the intralymphatic injections being that the allergens are delivered directly at the site of the immune response. (**2**) Antigen-presenting cells (APCs) take up the allergens (**3**) and bring them to the draining lymph node (only B). (**4**) In the lymph node, APCs present allergen peptides on MHC class II to naïve $CD4^+$ T helper cells. (**5**) Subsequently, naïve $CD4^+$ T helper cells differentiate into T helper type 1 (Th1) cells and/or T regulatory cells (Tregs). (**6**) The Th1 cells produce **IFN-γ**, and instruct the B cells to undergo class switching, (**7**) and start producing **IgG** and **IgA** antibodies. (**8**) Additionally, Treg cells produce regulatory cytokines **IL-10** and TGFβ

effective in human patients [29] with the advantage that it can be applied by the patients at home, reducing the number of visits to the doctor [29]. We are presently developing a version of SLIT for horses, where a porridge of transgenic barley expressing *Culicoides* allergens is brought in contact with the oral mucosa using a special bit [52••]. This system has the advantage that the barley grains are used directly, thus bypassing protein purification, which is cumbersome and expensive. In a pilot study, four horses were treated 6 times with this transgenic barley over a period of 20 weeks. After the treatment, a weak IgG1 and IgG4/7 antibody response was seen. Following one boost 8 months after the last treatment, a stronger allergen-specific IgG1 and IgG4/7 antibody response was achieved and the serum had some blocking activity. Allergen-specific antibodies could also be detected in the saliva. This pilot experiment suggests that this approach might be a useful option for ASIT in horses. Transgenic barley expressing various *Culicoides* allergens is now being produced with the aim to perform clinical trials in IBH-affected horses.

Therapeutic Vaccine Against IL-5

Eosinophils play a significant role in the pathogenesis of IBH, making them important therapeutic targets. Besides accumulating in IBH skin lesions, eosinophil counts were also found to be enhanced in peripheral blood. In fact, blood eosinophils were shown to correlate to IBH severity score [24••]. In humans affected with hyper-eosinophilic conditions, humanized monoclonal antibodies (mAb) targeting the IL-5 or IL-5 receptor α have proven to be safe and beneficial for the patients [60, 61]. This approach, however, will probably not be available for equine patients due to high production costs. A new approach has thus been developed: In contrast to passive immunization approach with mAb, horses were actively immunized against the eosinophilic master-molecule IL-5. Linkage of equine IL-5 to VLPs of the cucumber mosaic virus (CuMV) generated polyclonal antibody responses against IL-5 in vaccinated horses [24••, 25••]. Since this approach is limited by the horse's own immune system, effective antibody titer induction is ensured by the VLP design. Besides their small size of 20–200 nm that enables free draining into the lymphatic systems and mediates binding of native protein to B cell receptors, the VLP was optimized for effective stimulation of the innate and the adaptive immune system. The surface of the VLPs is highly repetitive allowing B cell receptor (BCR) cross-linking inducing potent antibody responses in absence of T cells. This leads to complement fixation via complement receptor 2 and subsequent follicular dendritic cell activation driving T cell–dependent differentiation of germinal center B cells into

class-switched affinity-matured long-lived plasma cells [62]. In addition, the VLP contains non-coding *E. coli* RNA activating innate immune receptors for pathogen-associated molecular patterns (PAMPs) such as toll-like receptor (TLR)-7 [63]. Finally, an integrated universal T cell epitope of tetanus toxin ($CuMV_{TT}$) suggests to boost the immune response towards the vaccine in all tetanus-vaccinated individuals [64]. Thus, the eIL-5-$CuMV_{TT}$ vaccine has been shown to induce effective antibody titers against IL-5 that led to significantly reduced numbers of eosinophils in blood of IBH-affected horses when comparing to placebo treatment (Fig. 3) [25••]. The first treatment year with two initial injections induced only short-lived antibody titers, hence a mid-season booster injection was required. However, once immunity was established by three basic vaccination shots, a single annual booster injection prior to IBH season was sufficient to maintain antibody titer throughout the following IBH season. The vaccine-induced anti-IL-5 antibody titers were shown to be reversible, an important safety criterion [25••]. Moreover, studies in mouse models indicate that the endogenous protein was not able to boost antibody titers in absence of VLP-linkage, because the induced immune response is dependent on the VLP-specific T cell help [65]. This represents an important autoimmunity safety criterion. If confirmed in a clinical setting, this would represent an advantage of the active vaccination over the passive mAb approach, where anti-therapeutic antibodies can be induced, limiting their use in affected patients [66]. Furthermore, the active immunization strategy described here requires substantially less frequent injections and the administered dose is significantly lower in the vaccine compared with doses of therapeutic mAb. With regards to clinical benefit, two placebo-controlled double-blind randomized clinical trials with IBH-affected Icelandic horses showed that the eIL-5-$CuMV_{TT}$ vaccine significantly reduced IBH lesion scores in line with decreased eosinophil blood counts. The IBH lesion reduction was more pronounced in the second treatment year due to more stable antibody responses [24••, 25••].

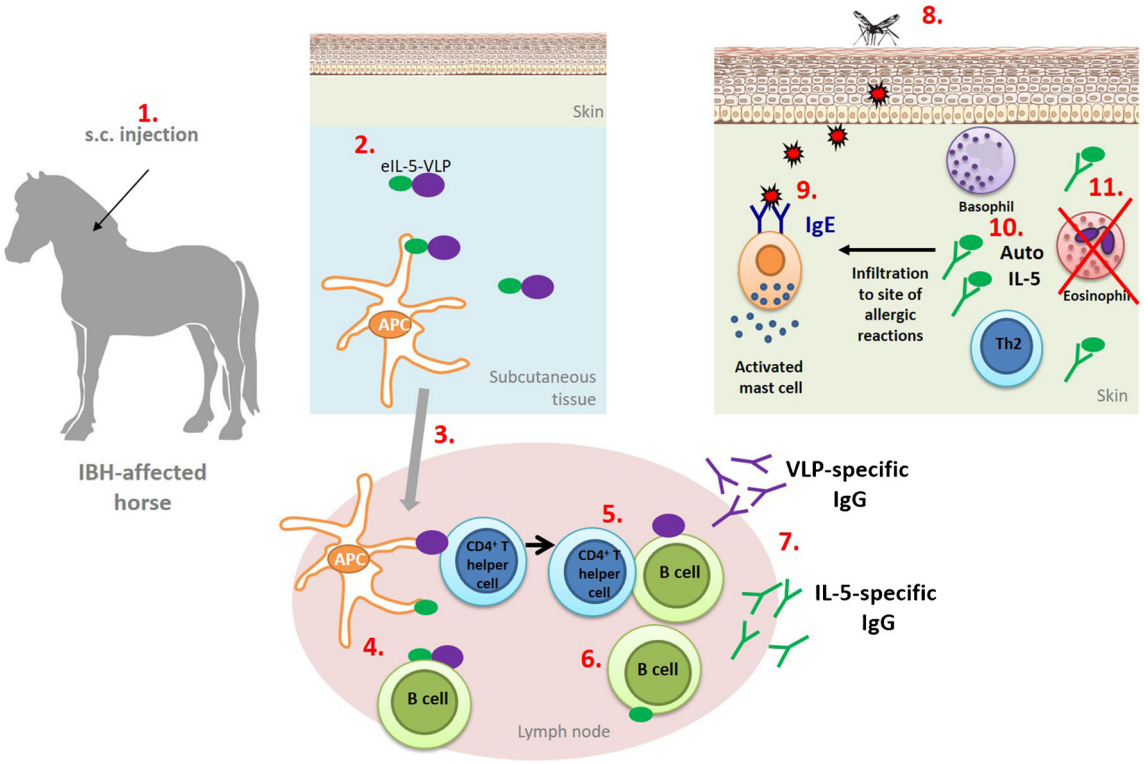

Fig. 3 Simplified scheme of the mechanism of the therapeutic vaccination against IBH with equine IL-5 virus–like particles (eIL5-VLPs). (**1**) The horses are injected subcutaneously with the vaccine, consisting of equine IL-5 linked to virus-like particles (eIL-5-VLPs). (**2**) Within subcutaneous tissue, part of the vaccine will be taken up by antigen-presenting cells (APCs) and (**3**) passive free drainage of vaccine (allowed by particle size) and active APC migration to the draining lymph node occurs. (**4**) APCs including B cells present antigens either natively or as epitopes on MHC class II to naïve $CD4^+$ T helper cell. (**5**) T cell help provided for the MHC class II–presented foreign VLP antigens induce class switching of VLP-specific B cells. (**6**) In parallel, eIL-5-specific B cells receive bystander T help leading to class switching of eIL-5-specific B cells (**7**) resulting in secretion of long-lived VLP- and IL-5-specific IgG antibodies. (**8**) When the allergic horse is bitten by *Culicoides*, the allergens are injected into the skin and (**9**) bind to mast cell–bound IgE, leading to an immediate type allergic reaction. (**10**) Because endogenous IL-5 is neutralized by the vaccine-induced antibodies, differentiation, infiltration, and activation of eosinophils are inhibited and reduce clinical sign of IBH

Therapeutic Vaccine Against IL-31

A second vaccine using the same $CuMV_{TT}$ VLP backbone but targeting equine IL-31 was generated by the same group. IL-31 is mainly a Th2 cell–derived cytokine that directly interacts with the nervous system via the IL-31 receptor expressed by dorsal root ganglia cells in the skin and thus triggers allergic pruritus [67, 68]. In IBH, it was shown that *Culicoides* allergen stimulation of peripheral blood mononuclear cells (PBMCs) produced significantly higher levels when derived from IBH-affected horses as compared with healthy horses. Moreover, IL-31 was shown to be exclusively expressed in punch biopsies of IBH-affected skin lesions, whereas it was not detected in non-lesional skin of the same horses or in skin of healthy horses [69••]. A first placebo-controlled randomized double-blind study suggested clinical benefit by reduction of IBH lesion scores in the vaccine group over the placebo group [69••]. Further studies including larger patient cohorts and combining both IL-5 and IL-31 vaccines will elucidate particular benefit of the vaccines for specific subgroups of IBH-affected horses.

Other Treatment Options for IBH

Anecdotal reports indicated that treatment of horses with a vaccine against dermatophytosis reduced clinical signs of IBH, possibly by redirecting the immune response from Th2 towards Th1. A placebo-controlled study could, however, not demonstrate a significant improvement of clinical signs after three injections of Insol®-Dermatophyton [70]. Interestingly, an increase in serum IFN_{λ}, TNFα and IL-10 could only be observed in the treated but not in the placebo group, indicating the induction of a Th1/Treg immune response [70].

Additionally, topical treatments can also alleviate clinical signs of IBH. A cream containing omega-3-fatty acids, humectants, and emollients has been shown to improve clinical lesions of IBH, but did not influence the pruritic score [71].

JAK kinase inhibitors, as used for treatment of atopic dermatitis in dogs [72], are not registered for treatment of horses. Nevertheless, anecdotal reports indicate that the JAK kinase inhibitor oclacitinib may have some effect for symptomatic treatment of IBH.

Conclusions

Biologicals are increasingly used for treatment of allergy, and the IL-5 and IL-31 therapeutic vaccines represent innovative advances for symptomatic treatment of IBH. Further studies are now needed to determine for which patient groups the respective therapeutic vaccines are most suited and whether their combination might further improve clinical response.

The availability of a large panel of pure recombinant *Culicoides* allergens relevant for IBH open new options for preventive and therapeutic ASIT. Suitable application routes and adjuvants have been studies in horses not exposed to *Culicoides*. A clinical trial for testing the efficacy of a preventive ASIT needs to be performed. Finally, therapeutic ASIT with the pure *Culicoides* allergens and the newly developed immunization protocols can now be tested in IBH patients, ideally by selecting the relevant *Culicoides* allergens for each IBH patients, whereby the recently developed microarray represents an efficient tool for selection of the required *Culicoides* allergens.

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Compliance with Ethical Standards

Conflict of Interest Dr. Elaine Marti reports grants from the Swiss National Science Foundation and from the Morris Animal Foundation. Dr. Sigurbjörg Torsteinsdottir reports grants from the Icelandic Research Fund, The Agricultural Productivity Fund of Iceland, and The University of Iceland Research Fund. Dr. Antonia Fettelschoss-Gabriel is involved in the development of active immunotherapies. Dr. Fettelschoss-Gabriel also received grants and fees from the University of Zurich and Evax AG. Drs. Iva Cvitas, Sigridur Jonsdottir, and Vilhjálmur Svansson declare no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

References

Papers of particular interest, published recently, have been highlighted as:
- • Of importance
- •• Of major importance

1. Littlewood JD. Clinical manifestations of *Culicoides* hypersensitivity. In: Noli C, Foster A, Rosenkrantz W, editors. Veterinary allergy. 1st ed. Hoboken: Wiley; 2014. p. 287–90.
2. Riek RF. Studies on allergic dermatitis ("Queensland itch") of the horse. Aust Vet J. 1953;29(7):177–84. https://doi.org/10.1111/j.1751-0813.1953.tb13937.x.
3. Brostrom H, Larsson A, Troedsson M. Allergic dermatitis (sweet itch) of Icelandic horses in Sweden: an epidemiological study. Equine Vet J. 1987;19(3):229–36.

4. Kleider N, Lees MJ. *Culicoides* hypersensitivity in the horse: 15 cases in southwestern British Columbia. The Canadian veterinary journal = La revue veterinaire canadienne. 1984;25(1):26–32.
5. Fadok VA, Greiner EC. Equine insect hypersensitivity: skin test and biopsy results correlated with clinical data. Equine Vet J. 1990;22(4):236–40.
6. Quinn PJ, Baker KP, Morrow AN. Sweet itch: responses of clinically normal and affected horses to intradermal challenge with extracts of biting insects. Equine Vet J. 1983;15(3):266–72.
7. Schaffartzik A, Hamza E, Janda J, Crameri R, Marti E, Rhyner C. Equine insect bite hypersensitivity: what do we know? Vet Immunol Immunopathol. 2012;147(3-4):113–26. https://doi.org/10.1016/j.vetimm.2012.03.017.
8. Steinman A, Peer G, Klement E. Epidemiological study of *Culicoides* hypersensitivity in horses in Israel. The Veterinary record. 2003;152(24):748–51.
9. van Grevenhof EM, Ducro B, Heuven HC, Bijma P. Identification of environmental factors affecting the prevalence of insect bite hypersensitivity in Shetland ponies and Friesian horses in The Netherlands. Equine Vet J. 2007;39(1):69–73.
10. Wilson AD, Harwood LJ, Bjornsdottir S, Marti E, Day MJ. Detection of IgG and IgE serum antibodies to *Culicoides* salivary gland antigens in horses with insect dermal hypersensitivity (sweet itch). Equine Vet J. 2001;33(7):707–13.
11. Hellberg W, Wilson AD, Mellor P, Doherr MG, Torsteinsdottir S, Zurbriggen A, et al. Equine insect bite hypersensitivity: immunoblot analysis of IgE and IgG subclass responses to *Culicoides nubeculosus* salivary gland extract. Vet Immunol Immunopathol. 2006;113(1-2):99–112. https://doi.org/10.1016/j.vetimm.2006.04.009.
12. Eberl G, Colonna M, Di Santo JP, McKenzie AN. Innate lymphoid cells. Innate lymphoid cells: a new paradigm in immunology. Science (New York, NY). 2015;348(6237):aaa6566. https://doi.org/10.1126/science.aaa6566.
13. Camelo A, Rosignoli G, Ohne Y, Stewart RA, Overed-Sayer C, Sleeman MA, et al. IL-33, IL-25, and TSLP induce a distinct phenotypic and activation profile in human type 2 innate lymphoid cells. Blood Adv. 2017;1(10):577–89. https://doi.org/10.1182/bloodadvances.2016002352.
14. Galli SJ, Tsai M, Piliponsky AM. The development of allergic inflammation. Nature. 2008;454(7203):445–54. https://doi.org/10.1038/nature07204.
15. Abbas AK, Lichtman AH, Pillai S. Allergy. In: Abbas AK, Lichtman AH, Pillai S, editors. Cellular and molecular immunology. 9th ed. Philadelphia, USA: Elsevier; 2018. p. 437–57.
16. Hamza E, Doherr MG, Bertoni G, Jungi TW, Marti E. Modulation of allergy incidence in icelandic horses is associated with a change in IL-4-producing T cells. Int Arch Allergy Immunol. 2007;144(4):325–37. https://doi.org/10.1159/000106459.
17. Hamza E, Wagner B, Jungi TW, Mirkovitch J, Marti E. Reduced incidence of insect-bite hypersensitivity in Icelandic horses is associated with a down-regulation of interleukin-4 by interleukin-10 and transforming growth factor-beta1. Vet Immunol Immunopathol. 2008;122(1-2):65–75. https://doi.org/10.1016/j.vetimm.2007.10.018.
18. Hamza E, Akdis CA, Wagner B, Steinbach F, Marti E. *In vitro* induction of functional allergen-specific CD4+ CD25high Treg cells in horses affected with insect bite hypersensitivity. Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology. 2013;43(8):889–901. https://doi.org/10.1111/cea.12131.
19. Meulenbroeks C, van der Lugt JJ, van der Meide NM, Willemse T, Rutten VP, Zaiss DM. Allergen-specific cytokine polarization protects Shetland Ponies against *Culicoides obsoletus*-induced insect bite hypersensitivity. PLoS One. 2015;10(4):e0122090. https://doi.org/10.1371/journal.pone.0122090.
20. Wagner B, Miller WH, Morgan EE, Hillegas JM, Erb HN, Leibold W, et al. IgE and IgG antibodies in skin allergy of the horse. Vet Res. 2006;37(6):813–25. https://doi.org/10.1051/vetres:2006039.
21. Wilson AD. Immune responses to ectoparasites of horses, with a focus on insect bite hypersensitivity. Parasite Immunol. 2014;36(11):560–72. https://doi.org/10.1111/pim.12142.
22. Kurotaki T, Narayama K, Oyamada T, Yoshikawa H, Yoshikawa T. Immunopathological study on equine insect hypersensitivity ("kasen") in Japan. J Comp Pathol. 1994;110(2):145–52.
23. Pichler WJ. Delayed drug hypersensitivity reactions. Ann Intern Med. 2003;139(8):683–93.
24.•• Fettelschoss-Gabriel A, Fettelschoss V, Thoms F, Giese C, Daniel M, Olomski F, et al. Treating insect-bite hypersensitivity in horses with active vaccination against IL-5. The Journal of allergy and clinical immunology. 2018;142(4):1194-205.e3. https://doi.org/10.1016/j.jaci.2018.01.041 **Induction of anti-IL-5 antibodies following injection of eIL-5 linked to VLP to target eosinophils in IBH-affected horses resulting in significant reduction of clinical signs.**
25.•• Fettelschoss-Gabriel A, Fettelschoss V, Olomski F, Birkmann K, Thoms F, Buhler M, et al. Active vaccination against interleukin-5 as long-term treatment for insect-bite hypersensitivity in horses. Allergy. 2019;74(3):572–82. https://doi.org/10.1111/all.13659 **Follow-up study demonstrating that a yearly booster with eIL-5-VLP maintained the reduction of eosinophils in blood and clinical signs in IBH-affected horses.**
26. Marsella R. Equine allergy therapy: update on the treatment of environmental, insect bite hypersensitivity, and food allergies. The Veterinary clinics of North America Equine practice. 2013;29(3):551–7. https://doi.org/10.1016/j.cveq.2013.08.006.
27. Olsen L, Bondesson U, Brostrom H, Olsson U, Mazogi B, Sundqvist M, et al. Pharmacokinetics and effects of cetirizine in horses with insect bite hypersensitivity. Veterinary journal (London, England : 1997). 2011;187(3):347–51. https://doi.org/10.1016/j.tvjl.2009.12.030.
28. Fujita H, Soyka MB, Akdis M, Akdis CA. Mechanisms of allergen-specific immunotherapy. Clin Transl Allergy. 2012;2(1):2. https://doi.org/10.1186/2045-7022-2-2.
29. Devillier P, Molimard M, Ansolabehere X, Bardoulat I, Coulombel N, Maurel F, et al. Immunotherapy with grass pollen tablets reduces medication dispensing for allergic rhinitis and asthma: a retrospective database study in France. Allergy. 2019;74(7):1317–26. https://doi.org/10.1111/all.13705.
30. Noon L. Prophylactic inoculation against hay fever. Lancet. 1911;177(4580):1572–3. https://doi.org/10.1016/S0140-6736(00)78276-6.
31. Akdis CA, Blaser K, Akdis M. Mechanisms of allergen-specific immunotherapy. Chem Immunol Allergy. 2006;91:195–203. https://doi.org/10.1159/000090282.
32. Bellinghausen I, Metz G, Enk AH, Christmann S, Knop J, Saloga J. Insect venom immunotherapy induces interleukin-10 production and a Th2-to-Th1 shift, and changes surface marker expression in venom-allergic subjects. Eur J Immunol. 1997;27(5):1131–9. https://doi.org/10.1002/eji.1830270513.
33. Akdis CA, Blesken T, Akdis M, Wuthrich B, Blaser K. Role of interleukin 10 in specific immunotherapy. J Clin Invest. 1998;102(1):98–106. https://doi.org/10.1172/jci2250.
34. Akdis M, Akdis CA. Mechanisms of allergen-specific immunotherapy: multiple suppressor factors at work in immune tolerance to allergens. J Allergy Clin Immunol. 2014;133(3):621–31. https://doi.org/10.1016/j.jaci.2013.12.1088.
35. Zhernov Y, Curin M, Khaitov M, Karaulov A, Valenta R. Recombinant allergens for immunotherapy: state of the art. Curr Opin Allergy Clin Immunol. 2019. https://doi.org/10.1097/aci.0000000000000536.

36. Curin M, Garib V, Valenta R. Single recombinant and purified major allergens and peptides: how they are made and how they change allergy diagnosis and treatment. Annals of allergy, asthma & immunology : official publication of the American College of Allergy, Asthma, & Immunology. 2017;119(3):201–9. https://doi.org/10.1016/j.anai.2016.11.022.
37. Patel HD, Chambliss JM, Gupta MR. Utility and comparative efficacy of recombinant allergens versus allergen extract. Curr Allergy Asthma Rep. 2017;17(9):63. https://doi.org/10.1007/s11882-017-0727-9.
38. Valenta R, Campana R, Marth K, van Hage M. Allergen-specific immunotherapy: from therapeutic vaccines to prophylactic approaches. J Intern Med. 2012;272(2):144–57. https://doi.org/10.1111/j.1365-2796.2012.02556.x.
39.• Campana R, Marth K, Zieglmayer P, Weber M, Lupinek C, Zhernov Y, et al. Vaccination of nonallergic individuals with recombinant hypoallergenic fragments of birch pollen allergen Bet v 1: Safety, effects, and mechanisms. The Journal of allergy and clinical immunology. 2019;143(3):1258–61. https://doi.org/10.1016/j.jaci.2018.11.011 **A prophylactic vaccination in non-allergic humans against birch pollen allergy with recombinant allergens resulted in mixed T cell responses and induced blocking IgG antibodies.**
40. Barbet JL, Bevier D, Greiner EC. Specific immunotherapy in the treatment of *Culicoides* hypersensitive horses: a double-blind study. Equine Vet J. 1990;22(4):232–5.
41. Anderson GS, Belton P, Jahren E, Lange H, Kleider N. Immunotherapy trial for horses in British Columbia with *Culicoides* (Diptera:Ceratopogonidae) hypersensitivity. J Med Entomol. 1996;33(3):458–66. https://doi.org/10.1093/jmedent/33.3.458.
42. Ginel PJ, Hernandez E, Lucena R, Blanco B, Novales M, Mozos E. Allergen-specific immunotherapy in horses with insect bite hypersensitivity: a double-blind, randomized, placebo-controlled study. Vet Dermatol. 2014;25(1):29–e10. https://doi.org/10.1111/vde.12092.
43. van der Meide NM, Savelkoul HF, Meulenbroeks C, Ducro BJ, Tijhaar E. Evaluation of a diagnostic ELISA for insect bite hypersensitivity in horses using recombinant Obsoletus complex allergens. Veterinary journal (London, England : 1997). 2014;200(1):31–7. https://doi.org/10.1016/j.tvjl.2013.12.004.
44. Golden DB, Langois J, Valentine MD, Kagey-Sobotka A, Lichtenstein LM. Treatment failures with whole-body extract therapy of insect sting allergy. Jama. 1981;246(21):2460–3.
45. Lee TH, Kay AB, Douglas AC, Kemeny M. The treatment of Hymenoptera sensitivity with whole body extract: a false sense of security. Lancet. 1981;318(8241):301. https://doi.org/10.1016/S0140-6736(81)90541-9.
46. Schaffartzik A, Marti E, Crameri R, Rhyner C. Cloning, production and characterization of antigen 5 like proteins from *Simulium vittatum* and *Culicoides nubeculosus*, the first cross-reactive allergen associated with equine insect bite hypersensitivity. Vet Immunol Immunopathol. 2010;137(1-2):76–83. https://doi.org/10.1016/j.vetimm.2010.04.012.
47. Schaffartzik A, Marti E, Torsteinsdottir S, Mellor PS, Crameri R, Rhyner C. Selective cloning, characterization, and production of the *Culicoides nubeculosus* salivary gland allergen repertoire associated with equine insect bite hypersensitivity. Vet Immunol Immunopathol. 2011;139(2-4):200–9. https://doi.org/10.1016/j.vetimm.2010.10.015.
48. Langner KF, Jarvis DL, Nimtz M, Heselhaus JE, McHolland LE, Leibold W, et al. Identification, expression and characterisation of a major salivary allergen (Cul s 1) of the biting midge *Culicoides sonorensis* relevant for summer eczema in horses. Int J Parasitol. 2009;39(2):243–50. https://doi.org/10.1016/j.ijpara.2008.06.008.
49. van der Meide NM, Roders N. Sloet van Oldruitenborgh-Oosterbaan MM, Schaap PJ, van Oers MM, Leibold W, Savelkoul HF, Tijhaar E. Cloning and expression of candidate allergens from *Culicoides obsoletus* for diagnosis of insect bite hypersensitivity in horses. Vet Immunol Immunopathol. 2013;153(3-4):227–39. https://doi.org/10.1016/j.vetimm.2013.03.005.
50. Peeters LM, Janssens S, Goddeeris BM, De Keyser K, Wilson AD, Kaufmann C, et al. Evaluation of an IgE ELISA with *Culicoides* spp. extracts and recombinant salivary antigens for diagnosis of insect bite hypersensitivity in Warmblood horses. Veterinary journal (London, England : 1997). 2013;198(1):141–7. https://doi.org/10.1016/j.tvjl.2013.06.010.
51. Jonsdottir S, Stefansdottir SB, Kristinarson SB, Svansson V, Bjornsson JM, Runarsdottir A, et al. Barley produced *Culicoides* allergens are suitable for monitoring the immune response of horses immunized with *E coli* expressed allergens. Veterinary immunology and immunopathology. 2018;201:32–7. https://doi.org/10.1016/j.vetimm.2018.05.005.
52.•• Jonsdottir S, Svansson V, Stefansdottir SB, Mantyla E, Marti E, Torsteinsdottir S. Oral administration of transgenic barley expressing a *Culicoides* allergen induces specific antibody response. Equine veterinary journal. 2017;49(4):512–8. https://doi.org/10.1111/evj.12655 **Treatment via mucosa of the mouth with transgenic barley expressing an allergen could induce allergen-specific response.**
53.• Marti E, Wang X, Jambari NN, Rhyner C, Olzhausen J, Perez-Barea JJ, et al. Novel in vitro diagnosis of equine allergies using a protein array and mathematical modelling approach: a proof of concept using insect bite hypersensitivity. Veterinary immunology and immunopathology. 2015;167(3-4):171–7. https://doi.org/10.1016/j.vetimm.2015.06.013 **A study demonstrating a protein array containing *Culicoides* allergens as an important tool for detection of allergen-specific IgE in IBH horses.**
54.•• Novotny EN. Component-resolved microarray analysis of IgE sensitization profiles to Culicoides recombinant allergens in horses with insect bite hypersensitivity.: Vetsuisse, University of Bern. 2019. **Protein array with all available *Culicoides* allergens used to reveal the most important allergens in IBH.**
55. Jóhannsson V. The life cycles of *Simulium vittatum* Zett. in Icelandic lake-outlets. SIL Proceedings, 1922-2010. 1988;23(4):2170–8. https://doi.org/10.1080/03680770.1987.11899870.
56. Bjornsdottir S, Sigvaldadottir J, Brostrom H, Langvad B, Sigurdsson A. Summer eczema in exported Icelandic horses: influence of environmental and genetic factors. Acta Vet Scand. 2006;48:3. https://doi.org/10.1186/1751-0147-48-3.
57. Torsteinsdottir S, Scheidegger S, Baselgia S, Jonsdottir S, Svansson V, Bjornsdottir S, et al. A prospective study on insect bite hypersensitivity in horses exported from Iceland into Switzerland. Acta Vet Scand. 2018;60(1):69. https://doi.org/10.1186/s13028-018-0425-1.
58.•• Jonsdottir S, Hamza E, Janda J, Rhyner C, Meinke A, Marti E, et al. Developing a preventive immunization approach against insect bite hypersensitivity using recombinant allergens: a pilot study. Veterinary immunology and immunopathology. 2015;166(1-2):8–21. https://doi.org/10.1016/j.vetimm.2015.05.002 **Intralymphatic injections of *Culicoides* allergens in Th1 focusing adjuvant resulted in an induction of favorable immune response in horses for protection against IBH.**
59.•• Jonsdottir S, Svansson V, Stefansdottir SB, Schupbach G, Rhyner C, Marti E, et al. A preventive immunization approach against insect bite hypersensitivity: intralymphatic injection with recombinant allergens in Alum or Alum and monophosphoryl lipid A. Veterinary immunology and immunopathology. 2016;172:14–20. https://doi.org/10.1016/j.vetimm.2016.02.017 **MPLA was important for induction of allergen-specific Th1/Treg immune**

responses in horses following vaccination with recombinant allergens in Alum/MPLA.

60. Legrand F, Klion AD. Biologic therapies targeting eosinophils: current status and future prospects. J Allergy Clin Immunol Pract. 2015;3(2):167–74. https://doi.org/10.1016/j.jaip.2015.01.013.
61. Roufosse F. Targeting the interleukin-5 pathway for treatment of eosinophilic conditions other than asthma. Front Med. 2018;5:49. https://doi.org/10.3389/fmed.2018.00049.
62. Gatto D, Pfister T, Jegerlehner A, Martin SW, Kopf M, Bachmann MF. Complement receptors regulate differentiation of bone marrow plasma cell precursors expressing transcription factors Blimp-1 and XBP-1. J Exp Med. 2005;201(6):993–1005. https://doi.org/10.1084/jem.20042239.
63. Jennings GT, Bachmann MF. The coming of age of virus-like particle vaccines. Biol Chem. 2008;389(5):521–36.
64. Zeltins A, West J, Zabel F, El Turabi A, Balke I, Haas S, et al. Incorporation of tetanus-epitope into virus-like particles achieves vaccine responses even in older recipients in models of psoriasis. Alzheimer's and cat allergy NPJ vaccines. 2017;2:30. https://doi.org/10.1038/s41541-017-0030-8.
65. Spohn G, Schori C, Keller I, Sladko K, Sina C, Guler R, et al. Preclinical efficacy and safety of an anti-IL-1beta vaccine for the treatment of type 2 diabetes. Molecular therapy Methods & clinical development. 2014;1:14048. https://doi.org/10.1038/mtm.2014.48.
66. Steenholdt C, Bendtzen K, Thomsen OØ, Ainsworth MA. Measurement of infliximab and anti-infliximab antibody levels can help distinguish maintenance versus loss of response. Gastroenterol Hepatol (N Y). 2012;8(2):131–4.
67. Sonkoly E, Muller A, Lauerma AI, Pivarcsi A, Soto H, Kemeny L, et al. IL-31: a new link between T cells and pruritus in atopic skin inflammation. J Allergy Clin Immunol. 2006;117(2):411–7. https://doi.org/10.1016/j.jaci.2005.10.033.
68. Mizuno T, Kanbayashi S, Okawa T, Maeda S, Okuda M. Molecular cloning of canine interleukin-31 and its expression in various tissues. Vet Immunol Immunopathol. 2009;131(1-2):140–3. https://doi.org/10.1016/j.vetimm.2009.03.014.
69.•• Olomski F, Fettelschoss V, Jonsdottir S, Birkmann K, Thoms F, Marti E, et al. Interleukin 31 in insect bite hypersensitivity – alleviating clinical symptoms by active vaccination against itch. Allergy. 2019; accepted. **A study demonstrating the importance of IL-31 in the pathogenesis of IBH and revealing IL-31 as an important target to neutralize in IBH for reduction of clinical signs.**
70. Gehlen H, Brunner M, Klier J, Reese S. Therapy of summer eczema using Insol® Dermatophyton - a field study. Pferdeheilkunde Equine Med. 2016;32(4):306–15.
71. Huhmann R, Mueller RS. A cream containing omega-3-fatty acids, humectants and emollients as an aid in the treatment of equine *Culicoides* hypersensitivity. Vet Dermatol. 2019;30(2):155–e46. https://doi.org/10.1111/vde.12728.
72. Gonzales AJ, Bowman JW, Fici GJ, Zhang M, Mann DW, Mitton-Fry M. Oclacitinib (APOQUEL((R))) is a novel Janus kinase inhibitor with activity against cytokines involved in allergy. J Vet Pharmacol Ther. 2014;37(4):317–24. https://doi.org/10.1111/jvp.12101.